R E M A R K S

The Office Action dated July 15, 1988 has been carefully reviewed.

Reconsideration of the application is respectfully requested.

Applicants are pleased to note that the claims have been deemed free of any art rejection.

Claims 1-7 and 11 were rejected under 35 U.S.C. 112, first paragraph, for alleged lack of enablement.

The Examiner alleged that the term "warm blood animals" is too broad. The Examiner further alleged that the specification was seen to be enabling only for "in vitro blood cells".

In finding applicants' in vitro tests results to be insufficient for purposes of enablement, the Examiner is calling for in vivo testing. However, there is presently no animal model available for evaluation of drug efficacy regarding treatment of AIDS. In this regard see the enclosed copies of the following articles:

(1) Dessrosiers, R.C. and Letvin, N.L. Rev. Infect. Dis. 9, 438 (1987);

(2) Morrow, W.J.W. et al. J. Gen. Virol. 68, 2253 (1987); and

(3) Weber, J.N. et al, British Medical Bulletin, Vol. 44, No. 1, 20, (1988).

In view of the above, the Examiner's insistent of in vivo testing is tantamount to a requirement of in vivo testing in humans. This is a very strict and unreasonable standard for the granting of patent claims, which ahve otherwise been deemed patentable by the Examiner.

Of importance is that nucleoside analogs, of the type in the subject application, which are active in humans are active in vitro.

Of record is a copy of U.S.P. 4,710,492, issued December 1, 1987 wherein the Examiner therein is the supervisor herein and wherein a similar method claim for a similar disclosure was allowed for the same applicants. Has the standard of patentability changed since December of 1987?

References for the correlation of in vitro activity of nucleoside analogues against human immunodeficiency virus, HIV, with clinical efficacy against AIDS have been made of record. Also described in articles made of record is in vitro activity data for a compound according to the invention called "D4T" and why this activity justifies consideration of D4T as a clinical candidate for the treatment of AIDS.

The two nucleoside analogues for which clinical efficacy has been established are AZT; Fischl et al, New England Journal of Medicine, 1987, 317, 185-191 and DDC; Yarchoan et al, Lancet, 1988, 76-81. The in vitro activity of AZT was first described by Mitsuya et al, Proceedings of the National Academy of Sciences USA, 1985, 82, 7096-7100 and that of DDC by Mitsuya and Broder, Proceedings of the National Academy of Sciences USA, 1986, 83, 1915-1922.

Copies of these articles are of record. AZT has been approved for use in humans.

The antiretroviral activity of D4T has been published by Professor Prusoff and Dr. Lin against murine leukemia virus, Lin et al, Journal of Medicinal Chemistry, 1987, 30, 440-444, and against HIV, Lin et al, Biochemical Pharmacology, 1987, 36, 2713-2718. Copies of these articles are of record.

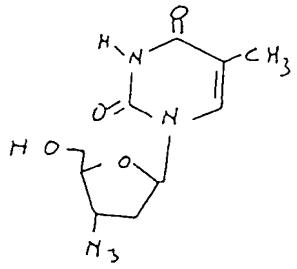
Also of record is the publication Herdewijn et al, Journal of Medicinal Chemistry, 1987, 30, 1270-1278, which is of interest because the authors provide in vitro results for D4T from two assay systems. In the first which utilizes ATH8 cells (Table II, p. 1273), AZT is slightly more active than D4T, but is also much more toxic; as a result, AZT has a poorer therapeutic index. DDC which inhibits the virus replication by 50% at only 0.2 micromolar is considerablly more active than either D4T or AZT. In contrast, the authors' second in vitro assay system which utilized MT4 cells (Table III, p. 1275) shows that D4T and AZT have comparable activity, and that both are considerably more potent than DDC. Because the results from different in vitro assay systems are variable, these types of experiments are use useful to identify compounds which have good potency against HIV, but are of limited utility to assign relative potency.

The above described Herdewijn et al paper states on page 1273 that the potency of D4T is comparable of that to AZT, but D4T is less toxic "which makes D4T a valuable candidate for further examination as a potential anti-HIV drug". They conclude this publication on page 1274 by indicating that it is "imperative to pursue"...D4T..." for

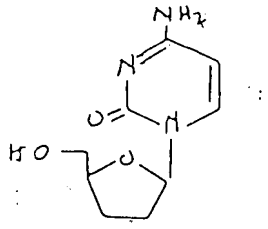
more extensive pharmacological studies in the scope of developing and appropriate chemotherapy for retrovirus infections (i.e., AIDS)."

The Examiner's statement that the core structures of the nucleoside (5-halo-3'-azido-2',3'-dideoxyuridine, Yarchoan et al.) are not similar to the isntant nucleoside is erroneous. This argument would lead to the rationale that the activity of 2',3'-dideoxycytidine (DDC) would probably not be active against AIDS in humans, although it was active in vitro. However, DDC is indeed active in humans against AIDS, although it has unpredicable toxicity.

The compound DDC is different in structure for AZT as seen below.



AZT



DDC

It is clear that DDC has no $N_3$, does have an $NH_2$ in place of O on carbon-4, and has no methyl moiety on carbon-5 of the pyrimidine moiety. Yet the two structures are similar in that they are both pyrimidine nucleosides and the parent compounds, thymidine and deoxycytidine, are natural components of viral DNA. Applicants' compounds are

also pyrimidine nucleoside analogs of thymidine and of deoxycytidine, as are AZT and DDC.

3'-Deoxythimidin-2'-ene(d4T) is similar to AZT, but lacks the $N_3$, and is unsaturated in the 2',3' position.

With the above in mind, reference is made to Cross v. Iizuka, 224 U.S.P.Q. 739 at 748 (Fed. Cir. 1985).

It is noted that Example 2 on pages 12-13 of the application concerns stimulated human peripheral blood mononuclear cells infected with HIV in the presence of 3-deoxythymidin-2'-ene. This example should be more than sufficient to satisfy the enablement requirement of 35 U.S.C. 112, first paragraph. The Examiner has not cited any case law to support her alleged need for in vivo testing.

The Examiner's attention is once again directed to two recent Board Decisions, namely Ex parte Chwang, 231 U.S.P.Q. 751 (Bd. App. & Int. 1986) (which cited Cross v. Iizuka, 224 U.S.P.Q. 739 (Fed. Cir. 1985)), Ex parte Krepelka et al, 231 U.S.P.Q. 746 and In re Hirsch, BNA PTCJ, Vol. 34, No. 850, October 8, 1987, pp. 588-589.

The Examiner is apparently trying to limit applicants only to their working examples and this is improper.

See In re Anderson, 176 USPQ 331, 333 (CCPA 1973), where the Court held that

> " we do not regard §112, first paragraph, as requiring a specific example of everything within the scope of a broad claim...What the Patent Office is here apparently attempting is to limit all claims to the specific examples, notwithstanding the disclosure of a broader invention. This it may not do."

> " It is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name ever such species. It is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it."

Indeed, examples per se are not required to satisfy the requirements of 35 U.S.C. 112, first paragraph. See In re Strahilevitz, 212 USPQ 561, 562-563 (CCPA 1982); In re Stephens, 188 USPQ 659, 660-662 (CCPA 1976); In re Borkowski, 57 CCPA 946, 164 USPQ 642, 645-646 (CCPA 1970); In re Gay, 50 CCPA 725, 135 USPQ 311, 316 (CCPA 1962).

The Court held in In re Robins, 166 USPQ 552, 555-556 (CCPA 1970) that working examples are only one means of satisfying the enablement requirement of 35 U.S.C. 112, and that the mere listing of specific compounds, chemical substituents, solvents, cross-linking agents, etc. in the specification would in most cases provide suitable evidence of enablement equivalent to specific working examples utilizing each of the various components.

The disclosure as set forth by the applicants in the application must be given the presumption of correctness and operativeness by the Patent and Trademark Office. The only relevant concern of the Patent and Trademark Office is the truth of the assertions in the application. In any event, the burden is on the Patent and Trademark Office whenever a rejection is made for lack of enablement under Section 112. The Examiner must explain why she doubts the truth or accuracy of the statements in a supporting disclosure to which the Examiner objects. The Examiner must back up such assertions with acceptable evidence or reasoning which contradicts applicants' contentions. See,

for example, In re Marzoocchi, 169 USPQ 367, 369-370 (CCPA 1967) and In re Bowen, 181 USPQ 48, 50-52 (CCPA 1974).

The Examiner in the case at hand has not carried her burden of showing the applicants' specification to be untrue or inaccurate; indeed, the Examiner gave no evidence or reasoning for the rejection.

Applicants do not believe that any experimentation would be necessary for one skilled in the art to practice their described invention. Assuming arguendo that a certain, limited degree of experimentation would be required for one skilled in that art to reproduce applicants' invention, such experimentation would not deter from applicants' satisfaction of the enablement requirement under 35 U.S.C. 112. See, for example, In re Miller, 169 USPQ 597, 602 (CCPA 1971); In re Angstadt, 190 USPQ 214, 218-219 (CCPA 1976); Ansul Company v Uniroyal, Inc., 179 USPQ 759, 763 (2d Cir. 1971), cert. denied, 172 USPQ 257 (1972); and Caldwell v. The United States, 175 USPQ 44, 47-48 (U.S. Ct. Cls. 1972).

It should be further noted that only those skilled in the art must be enabled, not the general public. In re Storrs, 114 USPQ 293, 296-297 (CCPA 1957).

Based on the above, applicants respectfully solicit withdrawal of the rejection of claims under 35 U.S.C. 112, first paragraph.

Applicants believe that this application is now in condition for allowance of all claims therein, and the early

issuance of a Notice of Allowance is respectfully requested.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Services as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on....OCTOBER 5, 1998....
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By.....

Date....OCTOBER 5, 1998....

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Key words: *HIV/infection/small animals*

# Small Animals Are Not Susceptible to Human Immunodeficiency Virus Infection

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SUMMARY

Several species of small animals were inoculated at birth or as adults with blood components from patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related disorders, or with the human immunodeficiency virus (HIV). No ill effects were noted in rats, hamsters, guinea-pigs, rabbits or musk shrews. Mice inoculated with clinical specimens had a significant incidence of mortality as compared with control groups (18·7% against 5·9%, $P < 0.025$). Mice receiving HIV showed an increase in mortality, but it was not statistically significant. Infection of the animals by HIV could not be detected by virological or immunological studies. We concluded that none of these animal species provided a useful model for evaluating HIV infection.

The acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus (HIV) (Coffin *et al.*, 1986), a retrovirus of the lentivirus subfamily. It preferentially replicates in human T cells, although it also infects B cells, macrophages and cells in the brain (Levy *et al.*, 1985*a*, *b*). Soon after its isolation and association with AIDS, its infectivity for chimpanzees was noted (Alter *et al.*, 1984; Francis *et al.*, 1984; Gajdusek *et al.*, 1984, 1985; Fultz *et al.*, 1986). No other animal species has shown susceptibility to the virus.

Since 1982, we have been attempting to induce AIDS in a variety of animal species to derive an economical animal model in which antiviral drugs and vaccines could be tested and immunopathological mechanisms investigated. For these studies, we initially used peripheral mononuclear cells (PMC) from patients with AIDS and AIDS-related conditions. After the isolation and characterization of the AIDS-associated retroviruses (ARV) (now termed $HIV_{SF}$) (Levy *et al.*, 1984), the AIDS virus itself was used in our experiments.

Newborns from several animal species were injected with AIDS-related material (Table 1). Rabbits and musk shrews (*Suncus murinus*) were also inoculated as adults. Shrews were studied because of their close phylogenetic relationship with simian species. Initially, the intraperitoneal (i.p.) or subcutaneous injections consisted primarily of Ficoll–Hypaque-separated PMC (approximately $10^6$ cells in 0·1 ml fluid per animal), but whole blood, platelets, plasma, ultracentrifuged plasma pellets and spleen cell suspensions were also used. These specimens came from patients with Kaposi's sarcoma, lymphadenopathy or idiopathic thrombocytopenic purpura. Some hamsters were also inoculated intracerebrally. Additionally, blind passages were conducted using lymphoid cells or tissue culture supernatants from inoculated animals. In subsequent studies, mice, rabbits and shrews were inoculated i.p. with high titred [$> 10^6$ c.p.m./ml of reverse transcriptase (RT) activity] $HIV_{SF2}$ and $HIV_{SF33}$. Control animals received cells from healthy, heterosexual volunteers or fluids from uninfected tissue culture cells.

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Table 1. *Lack of infection of small animal species with HIV*

| Animal* | Number | Age | Result |
|---|---|---|---|
| Mouse | 328† | NB‡ | —§ |
| Rat | 30 | NB | — |
| Hamster | 16 | NB | — |
| Guinea-pig | 14 | NB | — |
| Rabbit | 12 | NB | — |
| Rabbit | 6 | Adult | — |
| Musk shrew | 9 | Adult | — |

* The mice (BALB/c, ICR, WS, C3H/B1), rats (Fisher 344) and hamsters (Golden Syrian) were obtained primarily from Simonsen Laboratories, Gilroy, Ca. The MRL/*Mp-lpr/lpr* (MRL/*lpr*) mice were bred at the University of Southern California, Los Angeles, Ca.; they were obtained originally from Jackson Laboratories, Bar Harbor, Me. The guinea-pigs (Hartley) were supplied by Charles River Breeding Laboratories, Wilmington, Mass. and the rabbits (New Zealand White) by White Hare, Stark City, Mo. The shrews were obtained from Dr Gil Dryden, Slippery Rock University, Pa. and Dr Emilie Rissman, University of Texas, Austin, Tx.

† This number indicates animals that were inoculated with either AIDS-related material or with HIV. Details of control animals are given in Table 2.

‡ NB, Newborn.

§ The negative sign indicates no seroconversion or recovery of HIV from the animals.

The presence of HIV in the spleen, PMC, blood and serum of animals was measured in a standard cell culture system (Levy *et al.*, 1984, 1985*a*). Spleens from mice, rats, guinea-pigs and shrews were placed directly into culture in the presence of 10% human interleukin-2 (IL-2) (T cell growth supplement, Meloy Laboratories, Springfield, Va., U.S.A.). Blood samples (approximately 0·5 ml) from these animals were cultured directly in medium containing IL-2. Spleen cells from rabbits were separated on Ficoll–Hypaque gradients before being cultured. The spleen cells were stimulated for 3 days with phytohaemagglutinin (PHA) (Levy *et al.*, 1984) and then replated in the growth medium. Moreover, some cells were mixed with PHA-stimulated PMC from seronegative human donors. Serum (0·1 to 0·5 ml) from the animals was inoculated directly onto mitogen-stimulated human PMC obtained from normal HIV-negative volunteers (Levy *et al.*, 1985*b*). The supernatant fluid from all cultures was assayed for particle-associated RT activity (Hoffman *et al.*, 1985) every 3 days for up to 2 months. Antibodies to HIV in the animals were measured by an indirect immunofluorescence assay or an immunoblot procedure using HUT-78 cells chronically infected with $HIV_{SF2}$ (Kaminsky *et al.*, 1985; Pan *et al.*, 1987). At approximately 3 to 6 month intervals, blood smears were made from the inoculated mice and stained with 'Stat' stain (VWR, Brisbane, Ca., U.S.A.). Tissues from sacrificed or dead animals were fixed in formalin, and examined by standard procedures.

No unexplained mortalities or pathologies were observed in any of the 30 rats, 16 hamsters or 14 guinea-pigs inoculated with the clinical specimens. Moreover, none of the hamsters and guinea-pigs seroconverted. Sera from the rats were not available for testing. Twelve newborn and six juvenile rabbits inoculated with HIV showed neither seroconversion nor virus in their spleen cells or PMC. Similar results were obtained with shrews injected with HIV (six with $HIV_{SF2}$ and three with $HIV_{SF33}$) (Table 1).

In experiments with 300 mice (from approximately 50 litters of different strains), a significantly higher mortality was noted in the experimental animals receiving specimens from AIDS or ARC patients (18·7%), than in those receiving material from healthy controls (5·3%) ($P < 0{\cdot}025 > 0{\cdot}01$ chi-square test) (Table 2). Death appeared to be due to a variety of pathologies, including runting, tumours and opportunistic infections. The latter were indicated by chronic diarrhoea, pneumonitis and dermatological disorders. In some mice, fibrotic spleens were noted, but extracts of these tissues or fluids from the cultured splenic cells did not induce disease in recipient mice. Blood smears revealed numerous abnormalities in the test animals (Table 3), but the direct cause of these pathologies could not be determined. Neither infectious HIV nor antibodies to the virus could be detected in blood cultures or sera from any of the mice.

When $HIV_{SF2}$ was inoculated into six litters of newborn BALB/c and two litters of MRL/*lpr* mice, a higher mortality was noted in the experimental animals than in the control group (Table 2), but it was not statistically significant ($0{\cdot}3 < P \geqslant 0{\cdot}5$, chi-square test). Moreover, infectious

Table 2. *Deaths occurring in mice inoculated with clinical specimens from AIDS and ARC patients or with HIV*

| | Mouse strain | Deaths | |
|---|---|---|---|
| **Inoculations with clinical specimens** | | | |
| Experimental animals | BALB/c | 42/218* | 19.4%† |
| | C3H | 5/30 | 16.6%† |
| | ICR/WS | 7/41 | 17.0%† |
| | Total | 54/289 | 18.7%† |
| Control animals | BALB/c | 2/42 | 4.8% |
| | C3H | 0/18 | 0.0% |
| | ICR/WS | 2/15 | 13.3% |
| | Total | 4/75 | 5.3% |
| **Inoculations with HIV** | | | |
| Experimental animals | BALB/c | 5/33 | 15.1%‡ |
| | MRL/*lpr* | 0/6 | 0.0% |
| | Total | 5/39 | 12.8%‡ |
| Control animals | BALB/c | 1/16 | 6.2% |
| | MRL/*lpr* | ND§ | ND |
| | Total | 1/16 | 6.2% |

* The figures indicate the numbers of deaths that occurred in the total number of inoculated animals during the first 12 months of life. Losses due to attrition in the infancy period (4 weeks post-injection) are not included.

† Statistically significant ($P < 0{\cdot}025 > 0{\cdot}01$, chi-square test).

‡ Although the experimental group of animals had a higher incidence of mortality than the controls, this result was not statistically significant.

§ ND, Not done.

Table 3. *White cell abnormalities in blood smears from mice inoculated with AIDS-related material and control specimens*

| | Number expressing abnormalities | Abnormal neutrophil/lymphocyte ratio* | Eosinophilia | Monocytosis | Other |
|---|---|---|---|---|---|
| Experimental animals | | | | | |
| BALB/c | 18/35 | 4/35 | 5/35 | – | Basophilia, reticulocytosis, poikilocytosis, and atypical lymphocytes |
| ICR/WS | 7/21 | 0/21 | 4/21 | 2/21 | Reticulocytosis |
| C3H | 8/12 | 8/12 | 2/12 | 2/12 | Atypical lymphocytes |
| Controls | | | | | |
| BALB/c | 0/7 | – | – | – | – |
| ICR/WS | 1/6 | – | 1/6 | – | – |
| C3H | 0/3 | – | – | – | – |

* In 6-month-old mice, the normal ranges are approximately neutrophils, 10 to 25%; lymphocytes, 65 to 90%; eosinophils, 0·5 to 5%; monocytes, 0·5 to 5%.

HIV and seroconversion were not observed in these inoculated mice. Finally, in an attempt to adapt a variant of HIV to mice, $HIV_{SF2}$ was passaged at 2-week intervals, using two litters of animals (BALB/c) per passage. At each of seven passages, the animals were sacrificed, their spleens were removed and cultured for virus. One portion of cells was inoculated into a new group of newborn animals. Neither replicating HIV nor seroconversion was detected in any animals.

These results indicate that none of the species of animals tested was susceptible to infection by HIV, including the MRL/*lpr* mouse. This strain develops a massive lymphoproliferation of T cells (weakly staining LY $1^+$ cells which are associated with helper function) (Theofilopoulos & Dixon, 1985) that could possibly provide a large reservoir of target cells for HIV. In addition, newborn (immunonaïve) animals that are particularly susceptible to other experimental viral infections did not show signs of HIV infection. Finally, the failure to select a variant of HIV by serial passage in mice confirms that this animal species cannot be readily infected by this virus.

These observations *in vivo* mirror our results in cell culture systems in which purified PMC from a variety of different animal species were used to evaluate infection with two different strains of HIV (Levy *et al.*, 1985*a*): only cells from chimpanzees showed susceptibility. Transfection studies using an infectious DNA clone of HIV further suggested that in animal cells, a barrier to infection occurs at the cell surface, although intracellular regulation of HIV replication can also be demonstrated (Levy *et al.*, 1986). We cannot adequately explain the high incidence of mortality in the test mice as compared with the control groups. The deaths could have been due to a co-pathogen such as *Toxoplasma gondii* (Hofflin & Remington, 1985) injected with the human PMC preparations.

The authors wish to thank Drs Paul Volberding, Donald Abrams and James Delameter for providing many of the clinical specimens; Drs Gil Dryden and Emilie Rissman for supplying us with the shrews; Dr Kathelyn Steimer of the Chiron Corporation for performing some of the Western blots; Dr George Brecher for advice on the interpretation of certain blood smears and Ted Verdi, Okechukwu Ojogho, Isabelle Gaston and Denise Hardy for technical assistance. This work was supported by grants from the National Institute (U01-CA34980) and the State of California Universitywide Task Force on AIDS.

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addition of the tested analogue. The reciprocal of membrane resistance in chloride containing solution was assumed to be total membrane conductance ($G_m$) and the same parameter measured in chloride free solution was considered largely potassium conductance ($G_K$). The mean chloride conductance $G_{Cl}$ of each experimental group of fibers was estimated as the mean $G_m$ minus the mean $G_K$. Sodium and other small conductances were neglected. These determinations were made in several fibers of different preparations at three or more concentrations of each examined analogue in order to construct the dose–response curves for $G_{Cl}$. Moreover, for each compound the resultant conductances vs. concentration curves were fit to a single site binding equation with a nonlinear least-squares method from which the $IC_{50} \pm SD$ (concentration required for half-maximal $G_{Cl}$ block ± standard deviation) was determined. The excitability characteristics of the sampled fibers were determined intracellularly[15] at a different concentration of each compound by observing the intracellular membrane potential response recorded from one microelectrode to a square-wave constant-current delivered by a second microelectrode inserted within 100 μm from the voltage electrode. In each fiber the membrane potential was set by a steady holding current to −80 mV, before passing the depolarizing pulses.

For in vivo studies some of the tested compounds, thoroughly mixed in a bolus, were administrated in a single dose to different groups of rats. Electromyographic recordings were performed at different times, allowing sufficient time for the substance to distribute and for the effect to develop fully. Data are expressed as means ± SEM. Significance of differences between group means was calculated by the Student's $t$ test. The estimates for SEM of $G_{Cl}$ were obtained from the variances of $G_m$ and $G_K$, assuming no covariance, by standard methods.[17] Standard deviations for the $IC_{50}$ values were calculated from the variance–covariance matrix obtained during nonlinear fitting procedures.

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# 3′-Substituted 2′,3′-Dideoxynucleoside Analogues as Potential Anti-HIV (HTLV-III/LAV) Agents†

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A series of 2′,3′-unsaturated and 3′-substituted 2′,3′-dideoxynucleoside analogues of purines and pyrimidines have been synthesized and evaluated for their inhibitory activity against human immunodeficiency virus (HIV). The 2′,3′-unsaturated analogues of 2′,3′-dideoxycytidine (ddeCyd) and 2′,3′-dideoxythymidine (ddeThd), 3′-azido-2′,3′-dideoxythymidine (AzddThd), 3′-fluoro-2′,3′-dideoxythymidine, 2′,3′-dideoxycytidine (ddCyd), and 2′,3′-dideoxyadenosine (ddAdo) emerged as the most potent inhibitors of HIV-induced cytopathogenicity in the human T lymphocyte cell lines ATH8 and MT4. In ATH8 cells ddCyd, ddeCyd, and ddAdo had the highest therapeutic index whereas in MT4 cells AzddThd, ddThd, ddCyd, and ddAdo were the most selective. Derivatives from ddThd in which the substituent group was linked to the 3′-carbon atom via a thio, sulfonyl, or oxygen bridge were far less inhibitory to HIV than was AzddThd.

Acquired immunodeficiency syndrome (AIDS) is an immunosuppressive disease characterized by an immune impairment associated with life-threatening opportunistic infections and a high susceptibility to unusual forms of certain neoplasms (i.e., Kaposi's sarcoma).[1-3] Human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV), recently designated as human immunodeficiency virus (HIV),[4] has been recognized as the etiologic agent of AIDS.[5,6] Several compounds with different chemical structures (i.e., Suramin,[7-9] Evans Blue,[9] aurintricarboxylic acid,[10] HPA-23,[11,12] phosphonoformic acid,[9,13] ribavirin,[14] interferon-α,[15] AL-721,[16] and 2′,3′-dideoxyribonucleosides[17-20]) have been reported as having significant inhibitory effect against HIV in vitro. Of these compounds, 2′,3′-dideoxyribonucleosides have thus far proven to be the most potent antiretroviral agents in vitro.[17-20] Indeed, Mitsuya and co-workers reported that 3′-azido-2′,3′-dideoxythymidine (AzddThd) protected human T cells (clone ATH8) against the cytopathogenic effect of HIV at 1–5 μM,[17] and 2′,3′-dideoxycytidine (ddCyd) completely suppressed HIV-induced cytopathogenicity in vitro at 0.5 μM.[18] The 2′,3′-unsaturated analogue of ddCyd, ddeCyd, proved equally effective as ddCyd in protecting ATH8 cells against HIV,[19] while ddeThd, the 2′,3′-unsaturated analogue of 2′,3′-dideoxythymidine (ddThd), was

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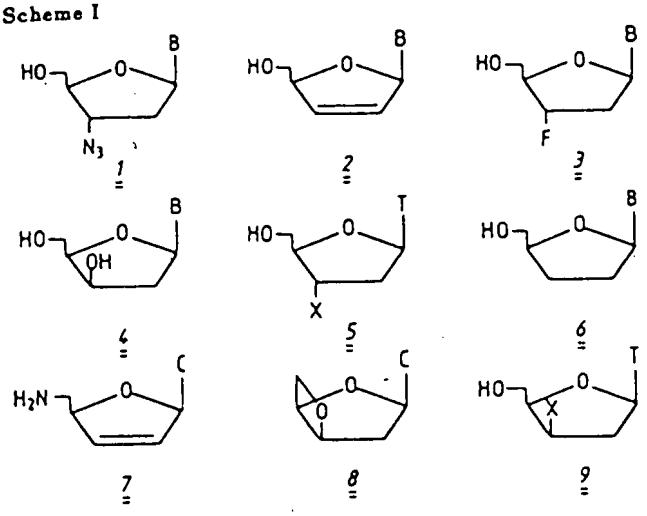
Scheme I



a, thymin-1-yl (T)
b, cytosin-1-yl (C)
c, adenin-9-yl (A)
d, uracil-1-yl (U)
e, guanin-9-yl (G)
f, 5-methylcytosin-1-yl
g, 5-ethyluracil-1-yl
h, $N^4$,5-dimethylcytosin-1-yl
i, 5-fluorouracil-1-yl
j, 5-iodouracil-1-yl
k, $N_3$
l, Cl
m, Br
n, I
o, OMe
p, OEt
q, $OCH_2COONa$
r, $OSO_2CH_3$
s, SEt
t, $SCH_2CH_2OH$
u, SCN

also found to be a potent and selective inhibitor of HIV.[20] Recently, Lin et al. reported on the antiviral activity of various 3′-azido, 3′-amino, 2′,3′-unsaturated, and 2′,3′-dideoxy analogues of pyrimidine deoxyribonucleosides against retroviruses.[21] From their studies, AzddThd, 5-bromo- and 5-iodo-3′-azido-2′,3′-dideoxyuridine, ddeThd, ddeCyd, and ddCyd emerged as the most active agents against Moloney murine leukemia virus.

The present study describes the synthesis of a series of nucleoside analogues and their anti-HIV activity, with two different target cell lines (ATH8 and MT4) for infection. From this study, several 2′,3′-dideoxythymidine and 2′,3′-dideoxycytidine derivatives emerged as potent and selective inhibitors of HIV-induced cytopathogenicity.

## Chemistry

3′-*O*-Methylthymidine (5o) and 3′-[(2-hydroxyethyl)-thio]-3′-deoxythymidine (5t) were synthesized by using the same modus operandi as described for the synthesis of 5p[22] and 5s,[23] respectively (Scheme I).

Although 9-(3-azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)adenine (1c) was previously synthesized by Imazawa[24] via a transglycosylation procedure, an alternative procedure was used here. The starting material for the synthesis of 1c, 9-[2-deoxy-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine, was synthesized by monomethoxytritylation of 4c.[25] However, difficulties were obtained during the workup procedure when the synthesis of 4c from 2′-*O*-tosyladenosine[25] was carried out in gram amounts. Therefore, we protected the 5′-hydroxyl function of 2′-*O*-tosyladenosine[26] by monomethoxytritylation (75%) before the product was subjected to the rearrangement conditions[25] (78%). Mesylation of 5′-protected 4c at the 3′-position, followed by reaction with sodium azide in dimethylformamide and deprotection with *p*-toluenesulfonic acid, afforded the 3′-azido compound 1c in satisfactory yield.

1-(3-Azido-2,3-dideoxy-β-D-*threo*-pentofuranosyl)thymine (9k) was obtained by 5′-deprotection with acetic acid of 1-(3-azido-2,3-dideoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine.[27] However, careful examination of the TLC (EtOAc) revealed 3′-azido-3′-deoxythymidine (1a) as a minor impurity. Both compounds can be separated by column chromatography with EtOAc as eluent.

3′-Thiocyanato-3′-deoxythymidine (5u) was synthesized by nucleophilic displacement of the mesyloxy group of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] with potassium thiocyanate, followed by deprotection with acetic acid. 3′-Deoxy-2′-thymidinene (2a) was isolated as the major compound in the reaction of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] with tetrabutylammonium fluoride in tetrahydrofuran, followed by detritylation. Reaction of 5′-*O*-trityl-3′-azido-3′-deoxythymidine[28] with 1-methylimidazole in the presence of phosphoryl chloride,[29] followed by a workup procedure with ammonia or methylamine and detritylation, afforded the 4-substituted 3′-azido-3′-deoxythymidine analogues 1f and 1h, respectively. The synthesis of 1f has been previously described;[30] in our procedure it was isolated as the HCl salt.

5-Ethyl-3′-azido-2′,3′-dideoxyuridine (1g) was synthesized from 5-ethyl-2′-deoxyuridine[31] according to a classical reaction sequence.[28,37] After protecting of the 5′-hydroxy


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Table I. $^1$H NMR[a] and $^{13}$C NMR[b] Spectral Data of 3′-Fluoro-2′,3′-dideoxynucleosides

| | H-1′[c] | $J_{1'F}$, $J_{1'F}$ | H-2′, H-2″ | H-3′ | $J_{3'F}$ | H-4′[d] | $J_{4'F}$ | H-5′, H-5″ |
|---|---|---|---|---|---|---|---|---|
| 3a | 6.22 | 7.9, 6.8 | 1.94–2.64 | 5.44 | 53.6 | 4.16 | 27.7 | 3.64 |
| 3g | 6.21 | 7.9, 6.7 | 2.03–2.68 | 5.30 | 53.6 | 4.16 | 27.7 | 3.64 |
| 3b | 6.25 | 8.6, 5.9 | 1.75–2.54 | 5.27 | 54.3 | 4.15 | 27.0 | 3.58 |
| 3c | 6.39 | 9.0, 5.7 | 2.50–3.30 | 5.44 | 53.4 | 4.25 | 26.8 | 3.62 |

| | C-1′ | C-2′ | $J_{C2'F}$ | C-3′ | $J_{C3'F}$ | C-4′ | $J_{C4'F}$ | C-5′ | $J_{C5'F}$ |
|---|---|---|---|---|---|---|---|---|---|
| 3a | 83.9 | 37.0 | 19.5 | 94.8 | 173.3 | 84.9 | 22.0 | 60.9 | 11.0 |
| 3g | 83.9 | 36.9 | 19.5 | 94.8 | 173.3 | 84.8 | 22.0 | 60.8 | 11.0 |
| 3b | 85.1 | 37.7 | 20.75 | 94.9 | 173.3 | 84.7 | 22.0 | 60.9 | 11.0 |
| 3c | 84.2 | 36.9 | 20.8 | 95.1 | 173.3 | 85.6 | 22.6 | 61.2 | 11.0 |

[a] All $^1$H NMR spectra were taken in $Me_2SO$-$d_6$ with $Me_4Si$ as internal standard; chemical shifts in δ values (ppm); coupling constants in hertz. [b] All $^{13}$C NMR spectra were taken in $Me_2SO$-$d_6$, which was used as internal standard (39.6 ppm); coupling constants in hertz. [c] H-1′ appears as a double doublet. [d] H-4′ appears as a doublet of broad triplets.

group by tritylation, the 3′-hydroxyl group of 5-ethyl-2′-deoxyuridine was mesylated with mesyl chloride in pyridine. Reversal of the configuration at the 3′-carbon atom was performed by refluxing in the presence of sodium hydroxide. This reaction proceeded via a 2,3′-anhydro intermediate. Mesylation of the 3′-hydroxyl group and nucleophilic displacement with azide, followed by detritylation, afforded 5-ethyl-3′-azido-2′,3′-dideoxyuridine in a total yield of 71% (starting from 5-ethyl-2′-deoxyuridine). 5-Ethyl-2′,3′-dideoxyuridine (6g) was synthesized from 5′-*O*-benzoyl-5-ethyl-2′-deoxyuridine according to a reaction sequence described by Prisbe.[32]

As mentioned before, when a solution of 1-(2,3-dideoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine in tetrahydrofuran was treated with tetrabutylammonium fluoride (TBAF) at room temperature, 5′-*O*-trityl-3′-deoxy-2′-thymidinene was formed as the major compound, with 5′-*O*-trityl-3′-fluoro-3′-deoxythymidine as the minor compound. After detritylation with 80% acetic acid, only 5% of 3′-fluoro-3′-deoxythymidine was isolated. The synthesis of 3′-fluoro-3′-deoxythymidine (3a) has been previously reported by Langen et al.[33,34] They described the opening of the 2,3′-anhydro bond of 2,3′-anhydro-1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine with HF–$AlF_3$ (28% yield) and the reaction of 3′-*O*-mesylthymidine with $KHF_2$[34] (14% yield). The same authors also synthesized 3′-deoxy-3′-fluorothymidine from 2,3′-anhydro-1-(2-deoxy-5-*O*-mesyl-β-D-*threo*-pentofuranosyl)thymine in a two-step procedure (total yield 28%).[33]

However, we wanted to investigate a more general procedure for the synthesis of 3′-fluoro-2′,3′-dideoxynucleosides, which would also be applicable to purine nucleosides. Therefore, the fluorinating agent (diethylamino)sulfurtrifluoride (DAST)[35] was reacted with four different 2′-deoxynucleosides. In all cases, the desired compounds were obtained in good to moderate yield. The reaction of 1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[36,37] with DAST in benzene–tetrahydrofuran gave 5′-*O*-trityl-3′-fluoro-3′-deoxythymidine in very good yield. Some destruction took place during the detritylation procedure, and the final yield of 3′-fluoro-3′-deoxythymidine (3a) was 62%. Only 3% of 3′-deoxy-2′-thymidinene could be isolated. The ratio of the amounts of 3a and 2a, obtained in the reaction with DAST, was opposite to the ratio obtained in the reaction with TBAF. The same reaction sequence (DAST in benzene, 80% HOAc) applied on 1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)-5-ethyluracil yielded 65% of 3′-fluoro-2′,3′-dideoxy-5-ethyluridine 3g.

The reaction of 1-(2-deoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)cytosine with DAST was less straightforward. 5′-*O*-Trityl-3′-fluoro-2′,3′-dideoxycytidine was obtained only in 36% yield. When the heterocyclic base was protected at $N^4$ with a benzoyl group, no significant amount of the 3′-fluoro compound could be isolated. 2′,3′-Dideoxy-3′-fluorocytidine 3b has been previously synthesized from 2′,3′-dideoxy-3′-fluorouridine via a multistep sequence in low yield.[33]

The purine nucleoside 9-[2,3-dideoxy-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine was treated with DAST in $CH_2Cl_2$. From this reaction mixture was isolated the 3′-fluoro compound 3c in 52% yield. Although some detritylation was observed, this reaction showed the usefulness of DAST in the presence of acid-labile groups (glycosidic bound and monomethoxytrityl ether). The observed detritylation may be partly responsible for the lower yield compared to that in the reaction with the uridine analogues (protected with a trityl group in place of a monomethoxytrityl group). Deprotection of the 5-hydroxyl group with 2% *p*-toluenesulfonic acid in dichloromethane–methanol gave 3′-fluoro-2′,3′-dideoxyadenosine 3c in 62% yield.

The $^1$H NMR and $^{13}$C NMR spectral data of the fluoro compounds are given in Table I. The almost identical chemical shifts and coupling constants obtained for the different compounds suggest that they possess a similar configuration.

Previously published methods were used to synthesize the following sugar-modified nucleosides: 3′-azido-3′-deoxythymidine[28] (1a), 3′-chloro-3′-deoxythymidine[38] (5l), 3′-bromo-3′-deoxythymidine[39,40] (5m), 3′-iodo-3′-deoxythymidine[41,42] (5n), 3′-(ethylthio)-3′-deoxythymidine[23] (5s), 3′-*O*-ethylthymidine[22] (5p), 3′-*O*-(carboxymethyl)thymidine[43,44] (5q), 3′-*O*-mesylthymidine[39] (5r), 1-(2-deoxy-3-*O*-mesyl-β-D-*threo*-pentofuranosyl)thymine[28] (9r), 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine[25] (4c), 1-(2-deoxy-β-D-*threo*-pentofuranosyl)thymine[36] (4a), 1-(2-deoxy-β-D-*threo*-pentofuranosyl)cytosine[45] (4b), 2′,3′-dideoxy-2′-cytidinene[46] (2b), 2′,3′-dideoxy-2′-uridinene[47] (2d),

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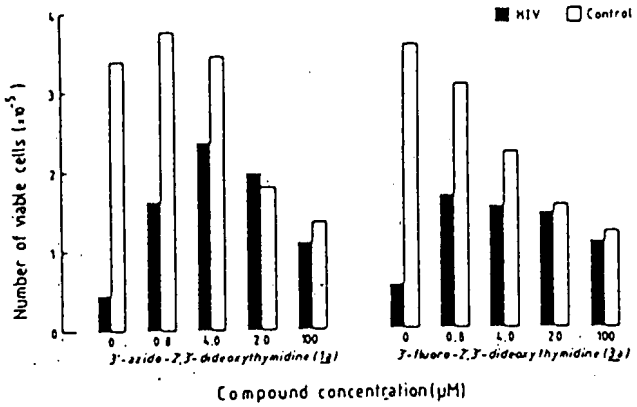


Figure 1. Inhibition of the cytopathogenicity of HIV for ATH8 cells by AzddThd and 3'-fluoro-ddThd. Viability of the cells was measured after an incubation period of 7 days (mock-infected cells incubated in the presence of different concentrations of the test compounds, □; HIV-infected cells incubated in the presence of different concentrations of the test compounds, ■).

2',3'-dideoxy-2'-adenosinene[48] (2c), 2',3'-dideoxyuridine[47] (6d), 1-(2-deoxy-3,5-epoxy-β-D-*threo*-pentofuranosyl)cytosine[46] (8), 5'-amino-2',3',5'-trideoxy-2'-cytidinene[49,50] (7), and 2',3'-dideoxythymidine[47] (6a).

## Antiviral Activity

The compounds evaluated for their anti-HIV effect can mainly be divided into five different structural classes: (i) 3'-substituted 2',3'-dideoxythymidine analogues; (ii) 2',3'-dideoxycytidine analogues, modified in the sugar and/or base moiety; (iii) 2'-deoxyxylo pyrimidine and 2'-deoxyxylo purine analogues; (iv) 3'-substituted and unsubstituted 5-ethyl-2',3'-dideoxyuridine analogues; and (v) 3'-substituted 2',3'-dideoxyadenosine analogues. Included in each class of compounds were the parental unmodified 2',3'-dideoxyribonucleosides and the corresponding 2',3'-didehydro-2',3'-dideoxyribonucleosides. Mitsuya and Broder, using an immortalized T cell line (ATH8) that is highly sensitive to the infectivity of HIV, found that various 2',3'-dideoxynucleosides (i.e., ddCyd, ddThd, ddAdo, ddIno, ddGuo), akin to the previously reported 3'-azido-ddThd (AzddThd),[17] inhibited both the cytopathogenicity and replication of HIV at doses that were not toxic to the host cells.[18] Among the 3'-substituted ddThd analogues reported in this paper, only 3'-fluoro (3a) and 3'-azido-ddThd (1a) were highly effective in protecting ATH8 cells against the cytopathic effect of HIV (Table II, Figure 1). However, 3a was more cytotoxic to the cells than AzddThd, and its in vitro therapeutic index, expressed as the ratio of compound concentration required to reduce the cell viability of normal uninfected ATH8 cells by 50% ($ID_{50}$) to the compound concentration required to achieve 50% protection of ATH8 cells against HIV ($ED_{50}$), was somewhat lower than that of AzddThd (10 and 19, respectively). None of the other 3'-halogeno derivatives of ddThd [i.e., 3'-chloro-, 3'-bromo-, 3'-iodo-ddThd (compounds 5l, 5m, 5n)] had a significant, if any, inhibitory effect against HIV ($ED_{50}$ > 100 μM). Also, substituents linked to the 3'-carbon of the sugar moiety of ddThd via a thio (i.e., 5s, 5t, 5u), sulfonyl (5r), or oxygen (i.e., 5o, 5p, 5q) bridge did not afford products capable of protecting

Table II. Comparative Potency and Selectivity of 2',3'-Dideoxyribonucleoside Analogues as Inhibitors of HIV Replication in ATH8 Cells

| compd | $ED_{50}$,[a] μM | $ID_{50}$,[b] μM | therapeutic index (ratio $ID_{50}/ED_{50}$) |
|---|---|---|---|
| 1a | 2.4 | 45 | 19 |
| 1c | 4.8 | 27 | 5.6 |
| 1f | >100 | >100 | |
| 1g | >100 | >100 | |
| 1h | >100 | >100 | |
| 2a | 4.1 | 110 | 27 |
| 2b | 0.30 | 30 | 100 |
| 2c | 40 | 52 | 1.3 |
| 2d | >100 | 107 | <1.1 |
| 3a | 1.4 | 15 | 10 |
| 3b | 8 | >250 | >31 |
| 3g | >500 | >500 | |
| 4a | >100 | >100 | |
| 4b | >100 | >100 | |
| 4c | >100 | >100 | |
| 5l | >500 | >500 | |
| 5m | >500 | 180 | <0.4 |
| 5n | >500 | >500 | |
| 5o | >100 | 88 | <0.9 |
| 5p | >100 | >100 | |
| 5q | >100 | >100 | |
| 5r | >100 | >100 | |
| 5s | >100 | 100 | <1 |
| 5t | >100 | >100 | |
| 5u | >100 | >100 | |
| 6a | 100 | >2000 | >20 |
| 6d | >500 | >500 | |
| 7 | 260 | >500 | >1.9 |
| 8 | 400 | >500 | >1.2 |
| 9r | >100 | >100 | |
| ddCyd | 0.20 | 35 | 175 |
| ddAdo | 2.7 | >500 | >148 |

[a] Effective dose of compound, achieving a 50% protection of ATH8 cells against the cytopathic effect of HIV. [b] Inhibitory dose of compound, required to reduce the viability of normal uninfected ATH8 cells by 50%.

ATH8 cells against HIV at concentrations lower than 100 μM. In contrast, the 2',3'-unsaturated derivative of ddThd, ddeThd (2a), proved markedly effective in inhibiting HIV-induced cytopathogenicity in ATH8 cells. Its $ED_{50}$ was comparable to that of AzddThd (4.1 μM and 2.4 μM, respectively), but it was less toxic for ATH8 cells than AzddThd ($ID_{50}$ = 110 μM and 45 μM, respectively), which makes ddeThd a valuable candidate for further examination as a potential anti-HIV drug in vivo.

Within the class of the ddCyd analogues, the 2',3'-unsaturated derivative of ddCyd, ddeCyd (2b), proved, like ddCyd itself, very effective as an inhibitor of HIV in vitro. Its effective antiviral dose was 0.30 μM, and its cytotoxic dose was 30 μM. More detailed investigations revealed that both compounds had comparable antiviral, antimetabolic, and cytostatic properties. 3'-Azido-5-methyl-ddCyd (1f) was totally devoid of anti-HIV activity when evaluated in the ATH8 cell system. However, deamination of 1f at the nucleoside level by Cyd/dCyd deaminase or at the nucleotide level by dCMP deaminase should convert this compound directly to the antiviral-active drug AzddThd or its 5'-monophosphate, AzddTMP. The fact that 1f was not antivirally active suggests that the compound is neither phosphorylated by dCyd kinase nor deaminated by Cyd/dCyd (or dCMP) deaminase. This is consistent with the observation that ddCyd is a poor substrate for human kidney Cyd/dCyd deaminase.[51] As

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(49) Adachi, T.; Arai, Y.; Inoue, I.; Saneyoshi, M. *Carbohydr. Res.* 1980, *78*, 67.
(50) Adachi, T.; Yamada, Y.; Inoue, I.; Saneyoshi, M. *Synthesis* 1977, 45.
(51) Kelly, J. A.; Litterst, C. L.; Roth, J. S.; Vistica, D. T.; Poplack, D. G.; Cooney, D. A.; Nadkarni, M.; Balis, F. M.; Broder, S.; Johns, D. G. *Drug Metab. Dispos.*, in press.

a consequence, 1f cannot be considered as a prodrug of AzddThd. Also, Mitsuya and Broder reported that 3′-azido-ddCyd was much less effective as an antiretroviral agent than ddCyd in ATH8 cells.[52] Hence, the total lack of antiretroviral activity of 1f and 1h is not surprising.

The fact that none of the 2′-deoxyxylo purine and 2′-deoxyxylo pyrimidine derivatives showed any protective effect against the HIV-infected ATH8 cells suggests that the 3′-hydroxyl in the "up" position is not compatible with the compound being recognized as a substrate for the enzymes involved in the metabolism (i.e., nucleoside and nucleotide kinases) or as an inhibitor for the enzyme (reverse transcriptase) involved in the final action of the compounds. The same is true for the 3′-epimer of AzddThd (9k), which is totally devoid of anti-HIV activity.

Of the ddUrd analogues tested [i.e., 3′-azido-5-ethyl-ddUrd (1g), 3′-fluoro-5-ethyl-ddUrd (3g), 2′,3′-didehydro-ddUrd (2d), ddUrd (6), and 3′-azido-5-methyl-ddUrd (1a)], none, except for 1a, showed any appreciable antiretroviral activity ($ED_{50}$ > 100 or even > 500 μM) (Table II). These data were confirmed with HIV-infected MT4 cells (Table III). For 3′-azido-5-ethyl-ddUrd (1g), our observations are in marked contrast with the data recently presented by R. Schinazi and co-workers at the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy (New Orleans, LA). (See also *Chem. Eng. News* 1986, *64*(49), 7–14.) They reported that 1g was equally active against HIV as AzddThd, but markedly less toxic than AzddThd when measured in a variety of normal cells (Vero, H9, peripheral mononuclear cells, fibroblasts). Compounds 1g, 3g, and 6g were not the only 5-substituted 2′,3′-dideoxyuridine analogues that were found inactive against HIV; other examples of inactive compounds include 1i and 1j (Mitsuya and Broder, unpublished data).

Among the ddAdo analogues tested, 3′-azido-ddAdo (1c) had similar antiretroviral activity as ddAdo ($ED_{50}$ = 3–5 μM) but was considerably more cytostatic. In contrast, the 2′,3′-unsaturated derivative of ddAdo, ddeAdo (2c), was much less active.

All compounds mentioned above were further evaluated in the human T4 cell line MT4.[53] These cells form macroscopically visible clusters upon cultivation at 37 °C. When clusters of uninfected or HIV-infected cells treated in the presence of an antivirally protective dose of the test compound are converted into a single cell suspension (by pipetting), they recluster within 4 h. Unprotected HIV-infected cells do not recluster. We compared the differential potencies of the test compounds against HIV-infected MT4 cells with those obtained in the ATH8 cell system. We found that within each class of compounds the order of antiretroviral potency was almost identical irrespective of whether the test system used was ATH8 or MT4 (Table III). However, depending upon the structural class of compounds evaluated, the concentrations at which the compounds showed a protective effect against HIV-infected MT4 cells varied considerably from those required to protect ATH8 cells against the cytopathic effect of HIV. The most striking differences were noted within the class of the 3′-substituted ddThd analogues. For example, AzddThd, ddeThd, and ddThd afforded 50% protection against HIV-infected MT4 cells at concentrations of 0.008 μM, 0.05 μM, and 1.25 μM, respectively, that is, 100–300-fold lower than the concentration required to protect HIV-infected ATH8 cells. The extremely high sensitivity of MT4 cells for the ddThd analogues was further confirmed by a cytopathogenicity assay in HIV-infected MT4 cells (data not shown). The biochemical basis for the differential activity of these compounds in both cell lines is now under investigation.

In conclusion, several analogues of ddThd and ddCyd (i.e., 2a, 2b, 3a, 3b) have been found to be potent inhibitors of HIV-induced cytopathogenicity in vitro. It seems imperative to pursue them for more extensive pharmacological studies in the scope of developing an appropriate chemotherapy for retrovirus infections (i.e., AIDS).

## Experimental Section

Melting points were determined in capillary tubes with a Büchi-Tottoli apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 257 spectrophotometer on samples in potassium bromide disks at 1.5%. Ultraviolet spectra were recorded with a Beckman UV 5230 spectrophotometer. Mass spectra were determined with an AEI MS-12 apparatus. The $^1H$ NMR and $^{13}C$ NMR spectra were determined with a JEOL FX 90Q spectrometer with tetramethylsilane as internal standard (s = singlet, d = doublet, t = triplet, br = broad signal, q = quadruplet, m = multiplet) unless stated otherwise. Precoated Merck silica gel F254 plates were used for TLC, and the spots were examined with UV light and sulfuric acid–anisaldehyde spray. Column chromatography was performed on Merck silica gel (0.063–0.200 mm). Anhydrous solvents were obtained as follows: tetrahydrofuran was obtained by distillation after reflux overnight with lithium aluminum hydride; pyridine was refluxed overnight in *p*-toluenesulfonyl chloride, distilled, refluxed overnight in potassium hydroxide, and distilled again; dichloromethane was stored for 1 week in anhydrous calcium chloride, filtered, and distilled; ethanol was dried by distillation after it had been refluxed overnight with magnesium–iodine; water was removed from *N,N*-dimethylformamide by distillation with benzene followed by distillation in vacuo; acetonitrile was first refluxed on phosphorus pentoxide and distilled; benzene was dried by distillation after it had been refluxed in the presence of sodium.

9-[2-Deoxy-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine. A. A mixture of 100 mg (0.4 mmol) of 9-(2-deoxy-β-D-*threo*-pentofuranosyl)adenine[25] and 149 mg (0.48 mmol) of 4-anisylchlorodiphenylmethane in 5 mL of anhydrous pyridine was stirred overnight. Methanol was added, and the reaction mixture was evaporated, diluted with $CHCl_3$ (20 mL), washed with $H_2O$ (2 × 20 mL), dried, evaporated, and coevaporated with toluene. The title compound was purified by column chromatography [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 95:5] and precipitated from $Et_2O$ (yield 72%): UV (MeOH) $\lambda_{max}$ 259 nm (ε 15 300); $^1H$ NMR ($CDCl_3$) δ 2.28–3.00 (m, 2 H, H-2′ and H-2″), 3.56 (m, 2 H, H-5′ and H-5″), 3.73 (s, 3 H, $CH_3$), 4.02 (m, 1 H, H-4′), 4.34 (m, 1 H, H-3′), 6.06 (dd, 1 H, *J* = 8.8 Hz and 2.6 Hz, H-1′), 6.51 (br s, 2 H, $NH_2$), 6.73 (d, 2 H, *o*-anisyl), 7.08–7.50 (m, 12 H, trityl), 7.87 and 8.14 (s, s, 1 H, 1 H, H-2 and H-8); $^{13}C$ NMR ($CDCl_3$) 40.6 (C-2′), 54.9 ($CH_3$), 62.5 (C-5′), 70.65 (C-3′), 84.0 and 84.4 (C-1′ and C-4′), 120.4 (C-5), 140.4 (C-8), 147.9 (C-4), 151.9 (C-2), 155.8 (C-6) ppm (values for the monomethoxytrityl group are not mentioned).

B. A mixture of 4.21 g (10 mmol) of 2′-*O*-tosyladenosine[26] and 4.01 g (13 mmol) of 4-anisylchlorodiphenylmethane in 100 mL of anhydrous pyridine was stirred overnight at room temperature. The reaction mixture was evaporated, diluted with $CHCl_3$ (250 mL), washed with water (2 × 250 mL), dried, evaporated, and coevaporated with toluene. Purification by column chromatography [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 98:2] afforded 5.2 g (75% yield) of 2′-*O*-tosyl-5′-*O*-(monomethoxytrityl)adenosine, which was precipitated from $CHCl_3$–$Et_2O$. Further elution of the column with $CHCl_3$–MeOH, 90:10, gave 500 mg (12%) of the starting material after crystallization from $H_2O$. UV (MeOH) $\lambda_{max}$ 260 nm (ε 12 800); $^1H$ NMR ($CDCl_3$) δ 2.36 (s, 3 H, $CH_3$-phenyl), 3.48 (m, 2 H, H-5′ and H-5″), 3.74 (s, 3 H, $CH_3O$), 4.32 (m, 1 H, H-4′), 4.76 (m, 1 H, H-3′), 5.12 (br s, 1 H, 3′-OH), 5.80 (dd, 1 H, H-2′), 6.14 (d, 1 H, *J* = 6.4 Hz, H-1′), 6.26 (br s, 2 H, $NH_2$), 6.85 (2 d, 4 H, *o*-anisyl and *m*-tosyl), 7.05–7.58 (m, 14 H, other aromatic protons), 7.83 and 8.04 (s, s, 1 H, 1 H, H-2 and H-8); $^{13}C$ NMR

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(53) Miyoshi, I.; Taguchi, H.; Kubonishi, I.; Yoshimoto, S.; Ohtsuki,

Table III. Toxicity and Inhibition of HIV Replication in MT4 Cells by Various 2',3'-Dideoxyribonucleoside Analogues

| | | concentration, μM | | | | |
|---|---|---|---|---|---|---|
| compd | assay | 125 | 5 | 0.2 | 0.008 | 0.00032 |
| 1a | Tox[a] | ++ | ++ | – | – | – |
| | Prot[b] | ND[c] | ND | +++ | ++ | + |
| 1c | Tox | ++ | + | – | ND | ND |
| | Prot | ND | ++ | – | – | ND |
| 1f | Tox | + | – | – | ND | ND |
| | Prot | +++ | ++ | + | ND | ND |
| 1g | Tox | ++ | – | – | ND | ND |
| | Prot | ND | + | – | – | – |
| 1h | Tox | – | – | – | ND | ND |
| | Prot | +++ | + | – | ND | ND |
| 2a | Tox | ++ | ++ | + | ND | ND |
| | Prot | ND | ND | +++ | + | – |
| 2b | Tox | ++ | – | – | ND | ND |
| | Prot | ND | +++ | ++ | – | ND |
| 2c | Tox | ++ | – | – | – | – |
| | Prot | – | – | – | ND | ND |
| 2d | Tox | ++ | – | – | ND | ND |
| | Prot | ND | – | – | – | ND |
| 3a | Tox | ++ | ++ | ++ | + | – |
| | Prot | ND | ND | ND | + | + |
| 3b | Tox | ++ | ++ | + | – | – |
| | Prot | ND | ND | ++ | – | – |
| 3c | Tox | – | – | – | ND | ND |
| | Prot | +++ | + | – | ND | ND |
| 3g | Tox | – | – | – | ND | ND |
| | Prot | + | – | – | ND | ND |
| 4a | Tox | – | – | – | ND | ND |
| | Prot | ++ | – | – | ND | ND |
| 4b | Tox | ++ | + | – | ND | ND |
| | Prot | ND | – | – | – | ND |
| 4c | Tox | + | – | – | ND | ND |
| | Prot | – | – | – | ND | ND |
| 5l | Tox | ++ | – | – | ND | ND |
| | Prot | ND | + | – | – | ND |
| 5m | Tox | ++ | – | – | ND | ND |
| | Prot | ND | + | – | – | ND |
| 5n | Tox | ++ | – | – | ND | ND |
| | Prot | ND | – | – | – | ND |
| 5o | Tox | ++ | + | – | ND | ND |
| | Prot | ND | + | – | – | ND |
| 5p | Tox | ++ | – | – | ND | ND |
| | Prot | ND | + | – | – | ND |
| 5q | Tox | – | – | – | ND | ND |
| | Prot | – | – | – | ND | ND |
| 5r | Tox | – | – | – | ND | ND |
| | Prot | +++ | + | – | ND | ND |
| 5s | Tox | ++ | – | – | ND | ND |
| | Prot | ND | + | – | – | ND |
| 5t | Tox | + | – | – | ND | ND |
| | Prot | + | – | – | ND | ND |
| 5u | Tox | ++ | – | – | ND | ND |
| | Prot | ND | + | – | – | ND |
| 6a | Tox | – | – | – | ND | ND |
| | Prot | ND | +++ | + | – | ND |
| 6d | Tox | – | – | – | ND | ND |
| | Prot | +++ | – | – | ND | ND |
| 6g | Tox | – | – | – | – | ND |
| | Prot | – | – | – | – | ND |
| 7 | Tox | + | – | – | ND | ND |
| | Prot | ++ | – | – | ND | ND |
| 8 | Tox | – | – | – | ND | ND |
| | Prot | + | – | – | ND | ND |
| 9k | Tox | – | – | – | ND | ND |
| | Prot | – | – | – | ND | ND |
| 9r | Tox | + | – | – | ND | ND |
| | Prot | +++ | – | – | ND | ND |
| ddCyd | Tox | ++ | – | – | ND | ND |
| | Prot | ND | +++ | ++ | – | ND |
| ddAdo | Tox | – | – | – | ND | ND |
| | Prot | +++ | ++ | – | ND | ND |

[a] Toxicity of the compound determined by evaluation of cluster morphology and "reclustering" properties in mock-infected MT4 cells (++, >90% toxicity; +, 10–40% toxicity; –, <10% toxicity). [b] Protection of MT4 cells against HIV replication determined by cluster morphology and "reclustering" properties (+++, >90% protection; ++, 40–60% protection; +, 10–40% protection; –, <10% protection). [c] Not determined.

($CDCl_3$) 21.3 ($CH_3$-phenyl), 55.0 ($CH_3O$), 63.1 (C-5'), 70.6 (C-3'), 79.8 (C-2'), 84.4 and 85.0 (C-4' and C-1'), 119.6 (C-5), 139.5 (C-8), 149.1 (C-4), 152.6 (C-2), 155.2 (C-6) ppm (values for the trityl carbon atoms are not mentioned).

To a solution of 4.16 g (6 mmol) of 2'-*O*-tosyl-5'-*O*-(monomethoxytrityl)adenosine in 60 mL of anhydrous THF, cooled in an ice bath, was added 60 mL of 1 M solution of lithiumn triethylborohydride in THF. After the reaction mixture was stirred overnight at room temperature, 10 mL of $H_2O$ was added and the reaction mixture was evaporated. The residue was diluted with $CH_2Cl_2$ (200 mL), washed with $H_2O$ (2 × 200 mL), dried, and evaporated, leaving an oil, which was purified by column chromatography ($CHCl_3$–MeOH, 97:3). The title compound was precipitated from $CHCl_3$–$Et_2O$ (yield 2.45 g, 4.7 mmol, 78%).

9-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-adenine (1c). Methanesulfonyl chloride (0.17 mL) was added to a solution of 366 mg (0.7 mmol) of 9-[2-deoxy-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine in 5 mL of anhydrous pyridine at 0 °C. The mixture was kept overnight in the refrigerator. After addition of 1 mL of $H_2O$, the reaction mixture was warmed up to room temperature, evaporated, diluted with $CHCl_3$ (20 mL), washed with $H_2O$ (2 × 20 mL), dried, and evaporated. Column chromatographic purification [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 97:3] gave 405 mg (0.67 mmol, 96%) of 9-(2-deoxy-3-*O*-mesyl-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl)adenine as a foam. The mesylation of the 3'-OH group was verified by NMR spectroscopy. $^1H$ NMR ($CDCl_3$) showed the appearance of a singlet at δ 2.71 ($CH_3SO_2$), a downfield shift for H-3' (δ 5.40), and a broad singlet at δ 6.54 ($NH_2$) as the only exchangeable protons. The $^{13}C$ NMR spectrum showed a strong downfield shift for C-3' (78.3 ppm) and the appearance of the methylsulfonyl group at 38.3 ppm. Sodium azide (100 mg) was added to a solution of 360 mg (0.6 mmol) of 9-[2-deoxy-3-*O*-mesyl-5-*O*-(monomethoxytrityl)-β-D-*threo*-pentofuranosyl]adenine in 5 mL of anhydrous DMF, and the reaction mixture was heated at 100 °C for 2 h. After cooling to room temperature and evaporation of the solvent, the mixture was taken up in $CHCl_3$ (20 mL) and washed with $H_2O$ (1 × 20 mL). The organic layer was dried and evaporated. TLC ($CHCl_3$–MeOH, 95:5, $R_f$ 0.44) revealed only one compound. This product was dissolved in 10 mL of $CH_2Cl_2$–MeOH (4:1) containing 2% *p*-toluenesulfonic acid and stirred for 15 min. After 0.25 mL of $Et_3N$ was added, the mixture was evaporated and applied onto a silica gel column, which was eluted with $CHCl_3$–MeOH (95:5). This yielded 100 mg (0.36 mmol, 60%) of 1c after crystallization from EtOH. IR, UV, $^1H$ NMR, and melting point were in agreement with those reported by Imazawa et al.:[24] mp 189–191 °C; IR (KBr) 2110 $cm^{-1}$ ($N_3$); UV (MeOH) $\lambda_{max}$ 259 nm (ε 15 600); $^1H$ NMR ($Me_2SO$-$d_6$) δ 2.32–2.66 (m, H-2'), 2.78–3.16 (m, H-2''), 3.60 (m, H-5' and H-5''), 3.95 (q, H-4'), 4.61 (m, H-3'), 5.34 (t, *J* = 5.7 Hz, OH), 6.32 (t, *J* = 6.6 Hz, H-1'), 7.32 (br s, $NH_2$), 8.15, 8.34 (2 s, H-2 and H-8).

3'-*O*-Methylthymidine (5o). 3'-*O*-Methylthymidine was synthesized by using exactly the same modus operandi as described by Hampton et al.[22] for the synthesis of 3'-*O*-ethylthymidine 5p: mp 133–135 °C (MeOH–$Et_2O$); MS, *m/e* 256 ($M^+$); UV ($H_2O$) $\lambda_{max}$ 266 nm (ε 9730), (0.2 N NaOH) $\lambda_{max}$ 265 nm (ε 7570); $^1H$ NMR ($Me_2SO$-$d_6$) δ 1.80 (s, 3 H, $CH_3$), 2.17 (m, 2 H, H-2' and H-2''), 3.28 (s, 3 H, $CH_3O$), 3.56 (m, 2 H, H-5' and H-5''), 3.90 (m, 2 H, H-3' and H-4'), 5.07 (t, 1 H, 5'-OH), 6.07 (dd, *J* = 7.9 Hz and 6.2 Hz, 1 H, H-1'), 7.68 (d, 1 H, H-6), 11.25 (br s, 1 H, NH). Anal. ($C_{11}H_{16}N_2O_5$) C, H, N.

3'-[(2-Hydroxyethyl)thio]-3'-deoxythymidine (5t). The same modus operandi as followed for the synthesis of the 3'-(ethylthio) derivative 5s[23] was used for the synthesis of 5t, except that 1-(2'-deoxy-3'-*O*-mesyl-5'-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] was used as starting material, ethanethiol was substituted by 2-mercaptoethanol, and the title compound (5t) was crystallized from $Et_2O$ (50% yield): mp 147 °C; MS, *m/e* 302 ($M^+$); UV (MeOH) $\lambda_{max}$ 266 nm (ε 10 100); $^1H$ NMR ($Me_2SO$-$d_6$–$D_2O$) δ 1.78 (d, 3 H, *J* = 1.3 Hz, $CH_3$), 2.10–2.56 (m, 2 H, H-2' and H-2''), 2.67 (t, 2 H, *J* = 6.7 Hz, $CH_2S$), 3.30–3.84 (m, 6 H, H-3', H-4', H-5', H-5'', and $CH_2O$), 6.04 (dd, 1 H, *J* = 6.6 Hz and 4.8 Hz, H-1'), 7.76 (d, 1 H, H-6). Anal. ($C_{12}H_{18}N_2O_5S$) C, H, N.

3'-Thiocyanato-3'-deoxythymidine (5u). A mixture of 996 mg (1.77 mmol) of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] and 777 mg (8 mmol) of potassium thiocyanate in 20 mL of anhydrous DMF was heated at 100 °C for 10 h. The reaction mixture was evaporated, diluted with $CHCl_3$ (50 mL), washed with $H_2O$ (2 × 50 mL), dried, and evaporated. The resulting oil was purified by column chromatography ($CHCl_3$–MeOH, 99:1), and the 5'-*O*-trityl derivative was detritylated with 20 mL of 80% acetic acid. After heating for 20 min at 100 °C, the solvent was evaporated and 3'-thiocyanato-3'-deoxythymidine (5u) was purified by column chromatography ($CHCl_3$–MeOH, 97:3) and crystallized from MeOH (105 mg, 0.37 mmol, 21%): mp 114–116 °C; UV ($H_2O$) $\lambda_{max}$ 266 nm (ε 9600), (0.1 N NaOH) $\lambda_{max}$ 266 nm (ε 8200); MS, *m/e* 283 ($M^+$), 252 ($M^+$ – $CH_2OH$), 158 ($M^+$ – $C_5H_6N_2O_2$), 126 ($C_5H_5N_2O_2$ + $H^+$), 99 (158 – HSCN); IR (KBr) 2150 $cm^{-1}$ (SCN); $^1H$ NMR (pyridine-$d_5$) δ 1.83 (s, 3 H, *J* = 0.88 Hz, $CH_3$), 2.82 (m, 2 H, H-2' and H-2''), 3.94–4.60 (m, 4 H, H-3', H-4', H-5', and H-5''), 6.63 (dd, 1 H, *J* = 6.6 Hz and 4.8 Hz, H-1'), 7.97 (d, 1 H, H-6), 13.1 (br s, 1 H, NH). Anal. ($C_{11}H_{13}N_3O_4S$) C, H, N.

3'-Azido-5-methyl-2',3'-dideoxycytidine (1f) and 1-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-4-(methylamino)-5-methyl-2(1*H*)-pyrimidinone (1h). A solution of 1.02 g (2 mmol) of 3'-azido-5'-*O*-trityl-3'-deoxythymidine[28] in 20 mL of a mixture of $CH_3CN$–pyridine (1:1) was added to a suspension containing 0.7 mL of phosphoryl chloride and 2 mL of *N*-methylimidazole in 15 mL of $CH_3CN$ at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was divided into two equal parts. To one part was added 10 mL of ammonia (33%). The other part was diluted with 10 mL of methylamine (50% in $H_2O$). After being stirred for 2 h at room temperature, both solutions were evaporated, diluted with $CHCl_3$ (100 mL), washed with $H_2O$ (2 × 100 mL), dried, and evaporated. The reaction products were purified by column chromatography ($CHCl_3$–MeOH, 98:2 and 99:1 for parts 1 and 2, respectively). Both compounds were fully characterized after detritylation with 80% acetic acid (100 °C, 20 min) and a second column chromatography ($CHCl_3$–MeOH, 95:5, and $CHCl_3$–MeOH, 97:3, respectively). a. 3'-Azido-5-methyl-2',3'-dideoxycytidine: 52% yield; UV ($H_2O$) $\lambda_{max}$ 275 nm (ε 9700); IR (KBr) 2090 $cm^{-1}$ ($N_3$); MS, *m/e* 266 $M^+$. Further identification was performed on the hydrochloride salt, which was obtained by treatment of a methanolic solution of the nucleoside with 1 N HCl in MeOH followed by addition of $Et_2O$ and crystallization: mp 176 °C dec; $^1H$ NMR ($D_2O$) δ 2.05 (d, 3 H, *J* = 0.7 Hz, $CH_3$), 2.55 (m, 2 H, H-2' and H-2''), 3.90 (m, 2 H, H-5' and H-5''), 4.06 (m, 1 H, H-4'), 4.34 (m, 1 H, H-3'), 6.16 (t, 1 H, *J* = 6.15 Hz, H-1'), 7.95 (d, 1 H, H-6). Anal. ($C_{10}H_{15}N_6O_3Cl$) C, H, N. b. 1-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-4-(methylamino)-5-methyl-2(1*H*)-pyrimidinone (53% yield) was precipitated from MeOH–$Et_2O$: UV (MeOH) $\lambda_{max}$ 272 nm (ε 9900); IR (KBr) 2100 $cm^{-1}$ ($N_3$); MS, *m/e* 280 ($M^+$); $^1H$ NMR ($Me_2SO$-$d_6$) δ 1.87 (d, 3 H, $CH_3$), 2.26 (t, 2 H, H-2' and H-2''), 2.81 (d, 3 H, *J* = 4.6 Hz, $CH_3NH$), 3.62 (m, 2 H, H-5' and H-5''), 3.83 (m, 1 H, H-4'), 4.35 (m, 1 H, $J_{3',4'}$ = 4.4 Hz, H-3'), 5.19 (br s, 1 H, OH), 6.11 (t, 1 H, *J* = 6.6 Hz, H-1'), 7.20 (q, 1 H, NH), 7.56 (d, 1 H, H-6). Anal. ($C_{11}H_{16}N_6O_3$) C, H, N.

1-(3-*O*-Mesyl-5-*O*-trityl-2-deoxy-β-D-*erythro*-pentofuranosyl)-5-ethyluracil. A mixture of 1 g (3.9 mmol) of 5-ethyl-2'-deoxyuridine[30] and 1.39 g (5 mmol) of trityl chloride in 50 mL of anhydrous pyridine was heated at 50 °C for 4 h and kept overnight at room temperature. The reaction mixture was cooled to 0 °C, mesyl chloride (1 mL) was added, and the reaction mixture was stored in the refrigerator overnight. After addition of $H_2O$ (1 mL), the solvent was evaporated, and the resulting oil was diluted with EtOAc (100 mL), washed with $H_2O$ (2 × 100 mL), dried, and evaporated. TLC analysis revealed mainly one compound ($CHCl_3$–MeOH, 97:3, $R_f$ 0.48), which was purified by flash chromatography on silica ($CHCl_3$–MeOH, 99:1) for $^1H$ NMR characterization (2.05 g, 3.56 mmol, 91% yield): $^1H$ NMR ($CDCl_3$) δ 1.86 (t, 3 H, *J* = 7.47 Hz, $CH_3CH_2$), 1.96 (dq, 2 H, $CH_2CH_3$), 2.54 (m, 2 H, H-2' and H-2''), 3.02 (s, 3 H, $CH_3SO_2$), 3.48 (m, 2 H, H-5' and H-5''), 4.31 (m, 1 H, H-4'), 5.38 (m, 1 H, H-3'), 6.40 (dd, 1 H, *J* = 5.9 Hz and 8.2 Hz, H-1'), 7.35 (m, 16 H, trityl and H-6).

1-(5-*O*-Trityl-2-deoxy-β-D-*threo*-pentofuranosyl)-5-ethyluracil. The foam obtained in the previous reaction was dissolved in EtOH (50 mL); 20 mL of 1 N NaOH and 50 mL of $H_2O$ were added, and the reaction mixture was refluxed. The

course of the reaction was followed by UV spectroscopy. During the reaction, the UV maximum changed first to a shoulder at 252 nm and then returned to 266 nm, which indicated the formation of a 2,3′-anhydro compound as an intermediate. After 5 h, the reaction mixture was cooled, concentrated to 60 mL, cooled in an ice bath, and acidified with 0.5 N HCl to pH 4. The precipitate was collected, washed thoroughly with $H_2O$, and dried in vacuo: 1.7 g (3.4 mmol, 96% yield); $R_f$ ($CHCl_3$–MeOH, 95:5) 0.58; UV (MeOH) $\lambda_{max}$ 266 nm (ε 10 100); $^1H$ NMR ($CDCl_3$) δ 1.91 (t, 3 H, $J$ = 7.47 Hz, $CH_2CH_3$), 1.98–2.60 (m, 4 H, $CH_2CH_3$, H-2′ and H-2″), 3.32–3.76 (m, 3 H, H-5′, H-5″, and OH), 4.09 (m, 1 H, H-4′), 4.40 (m, 1 H, H-3′), 6.17 (br d, 1 H, H-1′), 7.22 (m, 17 H, trityl and H-6), 10.07 (s, NH). Anal. ($C_{30}H_{30}N_2O_5$) C, H, N.

**3′-Azido-5-ethyl-2′,3′-dideoxyuridine (1g).** Mesyl chloride (1 mL) was added to a cooled (ice bath) solution of 1.7 g (3.4 mmol) of 1-(5-*O*-trityl-2-deoxy-β-D-*threo*-pentofuranosyl)-5-ethyluracil in 20 mL of anhydrous pyridine, and the reaction mixture was kept at 4 °C overnight and for an additional 5 h at room temperature. After addition of $H_2O$ (1 mL), the reaction mixture was evaporated, diluted with $CHCl_3$ (50 mL), washed with $H_2O$ (2 × 50 mL), dried, and evaporated, leaving an oil (one spot on TLC, $CHCl_3$–MeOH, 95:5, $R_f$ 0.65), which was dissolved in DMF (10 mL) and heated for 2 h at 100 °C in the presence of 1 g of sodium azide. After cooling to room temperature, the reaction mixture was poured into 250 mL of ice water; the precipitate was collected, washed with $H_2O$ (2 × 100 mL), dissolved in 50 mL of 80% acetic acid, and heated for 15 min at 100 °C. Evaporation, coevaporation with toluene, and chromatographic purification [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 98:2] yielded 787 mg (2.8 mmol, 82%) of 1g: mp (diisopropyl ether) 116–116.5 °C; MS, $m/e$ 281 ($M^+$); UV (MeOH) $\lambda_{max}$ 265 nm (ε 9680); IR (KBr) 2090 $cm^{-1}$ ($N_3^-$); $^1H$ NMR ($Me_2SO$-$d_6$) δ 1.04 (t, 3 H, $J$ = 7.47 Hz, $CH_3$), 2.22 (q, $CH_2CH_3$), 2.20–2.59 (m, H-2′ and H-2″), 3.61 (m, 2 H, H-5′ and H-5″), 3.83 (m, 1 H, H-4′), 4.41 (m, 1 H, H-3′), 5.21 (br t, 1 H, OH), 6.10 (t, 1 H, $J$ = 6.4 Hz, H-1′), 7.65 (s, 1 H, H-6), 11.25 (br s, 1 H, NH). Anal. ($C_{11}H_{15}N_5O_4$) C, H, N.

**5′-O-Benzoyl-5-ethyl-2′-deoxyuridine.** A solution of 0.48 mL (4 mmol) of benzoyl chloride in pyridine (25 mL) was added dropwise to a stirred solution of 1 g (3.9 nmol) of 5-ethyl-2′-deoxyuridine[31] in pyridine (25 mL) at 0 °C over a period of 1 h. MeOH was added, and, after being stirred for 10 min, the reaction mixture was evaporated and applied to a silica gel column eluting with $CHCl_3$–MeOH (95:5). The title compound was crystallized from MeOH–$Et_2O$: 1.1 g (3.05 mmol, 83%); mp 178–179 °C; UV (MeOH) $\lambda_{max}$ 266 nm (ε 11 000); $^1H$ NMR ($CDCl_3$–$CD_3OD$, 2:1) δ 0.91 (t, 3 H, $J$ = 7.47 Hz, $CH_3$), 2.12 (q, 2 H, $CH_2CH_3$), 2.28 (m, 2 H, H-2′ and H-2″, partly hidden by $CH_2CH_3$), 4.20–4.76 (m, 4 H, H-3′, H-4′, H-5′, and H-5″), 6.29 (t, 1 H, 6.8 Hz, H-1′), 7.12–7.64 (m, 4 H, H-6 and phenyl), 7.90–8.10 (m, 2 H, phenyl).

**5-Ethyl-2′,3′-dideoxyuridine (6g).** The modus operandi was exactly the same as used by Prisbe in ref 32 for the synthesis of 2′,3′-dideoxythymidine, except that the 3′-*O*-(methoxythio)-carbonyl derivative has not been crystallized and that tributyltin hydride was used instead of a mixture of bis(tributyltin) oxide and polymethylhydrosiloxane. The benzoyl group was removed with MeOH saturated with $NH_3$: total yield 77%; mp 117–118 °C; UV (MeOH) $\lambda_{max}$ 267 nm (ε 10 000); $^1H$ NMR ($CDCl_3$) δ 1.10 (t, 3 H, $J$ = 7.47 Hz, $CH_3$), 1.90–2.55 (m, 6 H, $CH_2CH_3$, H-2′, H-2″, H-3′ and H-3″), 3.82 (m, 2 H, H-5′ and H-5″), 4.12 (m, 1 H, H-4′), 6.08 (dd, 1 H, $J$ = 4.0 Hz and 6.6 Hz, H-1′), 7.48 (s, 1 H, H-6), 9.16 (br s, 1 H, NH); $^{13}C$ NMR ($CDCl_3$) δ 12.7 ($CH_3$), 20.1 ($CCH_3$), 25.2 (C-3′), 32.2 (C-2′), 63.4 (C-5′), 81.4 (C-4′), 86.4 (C-1′), 116.3 (C-5), 135.6 (C-6), 150.5 (C-2), 163.7 (C-4). Anal. ($C_{11}H_{16}N_2O_4$) C, H, N.

**1-(3-Azido-2,3-dideoxy-β-D-*threo*-pentofuranosyl)thymine (9k).** A solution of 1-(3-azido-2,3-dideoxy-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[27] (1.02 g, 2 mmol) in 80% acetic acid was heated for 15 min at 100 °C. The reaction mixture was evaporated, coevaporated with toluene, and isolated after two chromatographic purifications [(1) $CHCl_3$–MeOH, 95:5; (2) EtOAc], yielding the azidothymidine epimer 9k as a foam (300 mg, 56%). We did not succeed in crystallizing this compound: MS, $m/e$ 267 ($M^+$); IR (KBr) 2100 $cm^{-1}$ ($N_3$); UV (MeOH) $\lambda_{max}$ 267 nm (ε 9970); $^1H$ NMR ($Me_2SO$-$d_6$) δ 1.81 (s, 3 H, $CH_3$), 2.10 (m, 1 H, H-2′), 2.76 (m, 1 H, H-2″), 3.68 (m, 2 H, H-5′ and H-5″), 3.96 (m, 1 H, H-4′), 4.44 (m, 1 H, H-3′), 4.97 (br t, 1 H, OH), 6.01 (dd, 1 H, $J$ = 3.5 Hz and 7.5 Hz, H-1′), 7.48 (s, 1 H, H-6), 11.25 (br s, 1 H, NH); Anal. ($C_{10}H_{13}N_5O_4$) C, H, N.

**1-(5-*O*-Trityl-2-deoxy-β-D-*threo*-pentofuranosyl)cytosine.** A solution of 5.2 g (8 mmol) of 3′-*O*-mesyl-5′-*O*-trityl-*N*-benzoyl-2′-deoxycytidine in a mixture of EtOH (300 mL), $H_2O$ (100 mL), and 1 N NaOH (8 mL) was stirred overnight. After addition of another 50 mL of 1 N NaOH, the solution was heated at 100 °C for 3 h, cooled, and neutralized with 1 N HCl. After evaporation of ethanol, the precipitate was collected, washed twice with $H_2O$, and dried in vacuo (3.65 g, 94%): UV (MeOH) $\lambda_{max}$ 270 nm (ε 9400); $^1H$ NMR ($Me_2SO$-$d_6$) δ 1.81 (m, 1 H, H-2′), 2.53 (m, 1 H, H-2″), 3.28 (m, 2 H, H-5′ and H-5″), 4.14 (m, 2 H, H-3′ and H-4′), 5.13 (br d, 1 H, 3′-OH), 5.64 (d, 1 H, $J$ = 7.47 Hz, H-5), 6.06 (br d, 1 H, $J$ = 6.6 Hz, H-1′), 7.08 (br s, $NH_2$), 7.38 (m, 15 H, trityl), 7.65 (d, 1 H, H-6).

**1-(2′-Deoxy-β-D-*threo*-pentofuranosyl)cytosine[45] (4b).** This product was synthesized according to the method described by Ohtsuka et al.[45] However, the monomethoxytrityl instead of the trityl group was used as blocking group for the 5′-hydroxyl group. Detritylation was performed with 80% acetic acid at 100 °C for 15 min. The reaction mixture was chromatographed ($CHCl_3$–MeOH, 80:20), and the title compound was isolated as the hydrochloride (a solution of the nucleoside in $H_2O$ was acidified with 0.5 N HCl to pH 2, the water was evaporated, and the residue was crystallized from MeOH): UV ($H_2O$) $\lambda_{max}$ 272 nm (ε 9750) (for comparison with literature data,[14] the UV spectrum was taken before the nucleoside was converted to the hydrochloride salt); mp (decomposition started at 170 °C and was fast at 179 °C); $R_f$ ($CHCl_3$–MeOH–25% $NH_3$, 7:3:0.5) 0.48; $^1H$ NMR ($Me_2SO$-$d_6$) δ 2.03 (m, 1 H, H-2′), 2.38–2.72 (m, 1 H, H-2″), 3.74 (m, 2 H, H-5′ and H-5″), 3.90 (m, 1 H, H-4′), 4.43 (m, 1 H, H-3′), 5.96 (dd, 1 H, $J$ = 6.6 Hz, and 1.2 Hz, H-1′), 6.17 (d, 1 H, $J$ = 7.9 Hz, H-5), 8.13 (d, 1 H, H-6), 8.70 (br s, 1 H, NH), 9.73 (br s, 1 H, NH); $^{13}C$ NMR ($Me_2SO$-$d_6$) 40.8 (C-2′), 59.1 (C-5′), 68.2 (C-3′), 85.9 (C-1′), 86.4 (C-4′), 93.0 (C-5), 145.3 (C-6), 147.0 (C=O), 159.6 (C=O) ppm.

**3′-Deoxy-2′-thymidinene[47] (2a).** A solution of 500 mg (0.89 mmol) of 1-(2-deoxy-3-*O*-mesyl-5-*O*-trityl-β-D-*threo*-pentofuranosyl)thymine[28] in 10 mL of THF, containing 1 M TBAF, was stored at room temperature for 32 h. TLC of the reaction mixture revealed one major compound and two minor compounds (one of these being the starting material). After evaporation of the solvent, the reaction mixture was divided between $CHCl_3$ (50 mL) and $H_2O$ (50 mL). The organic layer was dried and evaporated. Column chromatographic purification ($CHCl_3$–MeOH, 98:2) gave only the major compound, 5′-*O*-trityl-3′-deoxy-2′-thymidinene,[48] in a pure form: $^1H$ NMR ($CDCl_3$) δ 1.28 (d, 3 H, $CH_3$), 3.39 (m, 2 H, H-5′ and H-5″), 4.96 (m, 1 H, H-4′), 5.88 (ddd, 1 H, $J$ = 6.0 Hz, 2.2 Hz, and 1.25 Hz, H-2′), 6.36 (ddd, 1 H, $J$ = 6.0 Hz and 2 × 1.75 Hz, H-3′), 7.05 (m, 1 H, H-1′), 7.27 (m, 16 H, trityl and H-6).

In a second preparation, the crude reaction mixture was stirred for 15 min in 80% acetic acid at 100 °C, evaporated, and coevaporated with toluene. TLC of the reaction mixture showed extensive destruction of the nucleosides. However, the two compounds that showed an UV absorption *and* a positive anisaldehyde–sulfuric acid test were isolated by column chromatography ($CHCl_3$–MeOH, 98:2) and identified as 3′-deoxy-2′-thymidinene (2a, 85 mg) and 3′-fluoro-3′-deoxythymidine (22 mg). 2a: mp 164–165 °C (lit.[16] mp 165–166 °C); UV ($H_2O$) $\lambda_{max}$ 265 nm (lit.[16] $\lambda_{max}$ 266 nm); $^1H$ NMR ($Me_2SO$-$d_6$) δ 1.75 (s, 3 H, $CH_3$), 3.60 (m, 2 H, H-5′ and H-5″), 4.76 (m, 1 H, H-4′), 4.98 (t, 1 H, 5′-OH), 5.92 (m, 1 H, H-2′), 6.39 (m, 1 H, H-3′), 6.81 (m, 1 H, H-1′), 7.62 (s, 1 H, H-6), 11.26 (br s, 1 H, NH).

**3′-Fluoro-3′-deoxythymidine (3a).** To a solution of 485 mg (1 mmol) of 1-(5-*O*-trityl-2-deoxy-β-D-*threo*-pentofuranosyl)thymine[36,37] in 20 mL of anhydrous benzene containing 1 mL of THF (solubility) was added 0.5 mL of DAST. The reaction mixture was stirred for 2 h, poured into a 5% solution of sodium bicarbonate (20 mL), and extracted with EtOAc (2 × 20 mL). TLC of the reaction mixture revealed mainly one compound. The organic layer was dried, evaporated, and treated for 15 min at 100 °C with 80% acetic acid. After evaporation, the reaction mixture was purified by column chromatography [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 99:1], yielding 7 mg (3%) of 3′-deoxy-2′-thymidinene (2a) and 152 mg (62%) of 3′-fluoro-3′-deoxythymidine (3a).

Melting point (176–177 °C) and spectroscopic data (UV, $^1$H NMR) were identical with those described in the literature:[33,34] $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.82 (s, 3 H, $CH_3$), 5.20 (br t, 1 H, 5′-OH), 7.71 (d, 1 H, $J$ = 1.1 Hz, H-6), 11.29 (br s, 1 H, NH); $^{13}$C NMR ($Me_2SO$-$d_6$) 12.2 ($CH_3$), 109.8 (C-5), 135.8 (C-6), 150.5 (C-2), 163.7 (C-4) ppm. (Values for the sugar protons and carbon atoms are presented in Table I.)

1-(3-Fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-5-ethyluracil (3g). DAST (1 mL) was added to a solution of 996 mg (2 mmol) of 1-(5-*O*-trityl-2-deoxy-β-D-*threo*-pentofuranosyl)-5-ethyluracil in 40 mL of anhydrous benzene. The reaction mixture was kept for 2 h at room temperature, washed with 20 mL of 5% sodium bicarbonate solution (2×), dried, and evaporated. This reaction mixture showed mainly one compound on TLC. Heating for 15 min at 100 °C in 80% acetic acid and evaporation of the solvent yielded an oil, which was applied onto a silica column and eluted with $CHCl_3$–MeOH (99.5:0.5) followed by $CHCl_3$–MeOH (98:2). 3g was crystallized from MeOH–$Et_2O$ (335 mg, 65%): mp 175–176 °C; UV (MeOH) $\lambda_{max}$ 266 nm ($\epsilon$ 9680); MS, $m/e$ 258 ($M^+$); $R_f$ ($CHCl_3$–MeOH, 90:10) 0.40; $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.02 (t, 3 H, $J$ = 7.47 Hz, $CH_2CH_3$), 2.22 (br q, $CH_2CH_3$), 5.18 (br t, 1 H, 5′-OH), 7.66 (d, 1 H, H-6), 11.27 (br s, 1 H, NH) (other values are presented in Table I); $^{13}$C NMR ($Me_2SO$-$d_6$) 12.7 ($CH_3$), 19.5 ($CH_2$), 115.4 (C-5), 135.0 (C-6), 150.2 (C-2), 163.1 (C-4) ppm (other values are presented in Table I). Anal. ($C_{11}H_{15}O_4N_2F$) C, H, N.

1-(3-Fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)cytosine (3b). DAST (0.5 mL) was added to a suspension of 485 mg (1 mmol) of 1-(5-*O*-trityl-2-deoxy-β-D-*threo*-pentofuranosyl)cytosine in a mixture of anhydrous $CH_2Cl_2$–THF (5:5, 10 mL). After the addition of DAST, the suspension cleared up immediately. The reaction mixture was stirred for 2 h at room temperature. After addition of $CHCl_3$ (10 mL), the mixture was poured into 20 mL of 5% $NaHCO_3$ solution. The organic layer was separated, and the water layer was extracted again with $CHCl_3$ (20 mL). The combined organic layer was dried, evaporated, and purified by column chromatography. The product with $R_f$ 0.32 ($CHCl_3$–MeOH, 90:10) was isolated (177 mg, 36%) and treated with 80% acetic acid for 15 min at 100 °C. Evaporation of the reaction mixture followed by column chromatography [(1) $CHCl_3$–MeOH, 95:5; (2) $CHCl_3$–MeOH, 90:10] and crystallization from MeOH afforded 49 mg (59% for the detritylation reaction) of 3b: mp 225 °C dec; UV ($H_2O$) $\lambda_{max}$ 271 nm ($\epsilon$ 9200), (0.01 N HCl) $\lambda_{max}$ 279 nm ($\epsilon$ 14 050); MS, $m/e$ 229 ($M^+$). Values not mentioned in Table I were as follows: $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 5.12 (br t, 1 H, 5′-OH), 5.74 (d, 1 H, $J$ = 7.47 Hz, H-5), 7.16 (br s, 2 H, $NH_2$), 7.76 (d, 1 H, $J$ = 7.47 Hz, H-6); $^{13}$C NMR ($Me_2SO$-$d_6$) 94.2 (C-5), 140.7 (C-6), 156.2 (C-2), 165.5 (C-4) ppm. Anal. ($C_9H_{12}O_3N_3F$) C, H, N.

9-(3-Fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)-adenine (3c). A mixture of 786 mg (1.5 mmol) of 9-[5-*O*-(monomethoxytrityl)-2-deoxy-β-D-*threo*-pentofuranosyl]adenine and 0.75 mL of DAST in 30 mL of anhydrous $CH_2Cl_2$ was kept for 1 h at room temperature and poured into 30 mL of 5% $NaHCO_3$ solution. The organic layer was dried, evaporated, and purified by column chromatography. 9-[5-*O*-(Monomethoxytrityl)-3-fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]adenine was eluted with $CHCl_3$–MeOH (98.5:1.5) (411 mg, 0.78 mmol, 52%): $^1$H NMR ($CDCl_3$) $\delta$ 2.61–3.27 (m, 2 H, H-2′ and H-2″), 3.41 (d, 2 H, $J$ = 4.4 Hz, H-5′ and H-5″), 3.78 (s, 3 H, $OCH_3$), 4.45 (dt, 1 H, $J_{4',F}$ = 26 Hz, H-4′), 5.36 (dm, 1 H, $J_{3',F}$ = 54.5 Hz, H-3′), 5.93 (br s, 2 H, $NH_2$), 6.44 (dd, 1 H, $J$ = 6.15 Hz and 8.35 Hz, H-1′), 6.79 (d, 2 H, $J$ = 9.2 Hz, *o*-phenyl), 7.25 (m, trityl), 7.93 and 8.23 (2 s, 2 × 1 H, H-8 and H-2). Detritylation was performed by treating the monomethoxytrityl compound (370 mg, 0.7 mmol) with 2% *p*-toluenesulfonic acid in $CH_2Cl_2$–MeOH (4:1) (20 mL) for 15 min at room temperature. After addition of 1.05 mL of 2 N NaOH, the reaction mixture was evaporated and purified by column chromatography [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 97:3]. The 3′-fluoro compound 3c was crystallized from MeOH (110 mg, 0.43 mmol, 62%): mp 188.5–190.0 °C; UV (MeOH) $\lambda_{max}$ 259 nm ($\epsilon$ 15 950); $R_f$ ($CHCl_3$–MeOH, 90:10) 0.45. Values not mentioned in Table I were as follows: $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 5.49 (t, 1 H, 5′-OH), 7.30 (s, 2 H, $NH_2$), 8.14 and 8.33 (2 s, 2 × 1 H, H-8 and H-2); $^{13}$C NMR ($Me_2SO$-$d_6$) 139.7 (C-8), 152.4 (C-2), 156.2 (C-6) ppm. Anal. ($C_{10}H_{12}N_5O_2F$) C, H, N.

Antiviral Test Procedures. HIV infection was carried out with the HTLV-III$_B$ strain. The virus was prepared from the culture supernatant of a persistently HTLV-III$_B$ infected H9 cell line. The antiviral assays, based on an inhibition of HIV-induced cytopathogenicity in human T4 lymphocyte (ATH8) cells, were carried out by following previously established procedures.[17,18]

The antiviral assays using MT4 as the target cell line were based on the MT4 cell cluster characteristics and "reclustering" properties. Briefly, MT4 cells were infected with HIV (300 $CCID_{50}$/well) and seeded at a density of 2.5 × $10^5$ cells/mL in the presence of varying concentrations of test compound. Five days after incubation at 37 °C, the cell cluster morphology of the MT4 cells was evaluated microscopically. Clusters of HIV-infected MT4 cells that had been protected by the compounds could be readily distinguished from HIV-infected MT4 cell clusters that had not been protected. These cell aggregates were then converted into a single cell suspension by pipetting and incubated at 37 °C for an additional 4 h, followed by microscopic assessment of the "reclustering" properties of the treated cell cultures. Mock-infected MT4 cells were evaluated under the same conditions. Reclustering of the mock-infected cells and cells treated with an antivirally protective dose of test compound occurred within a 4-h period. Nonprotected cells no longer reclustered. A more detailed description of the assay will be published elsewhere.

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